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COMPARATIVE ASSESSMENT OF APICAL MICROLEAKAGE AND DENTIN–SEALER INTERFACIAL ADAPTATION OF AH PLUS AND DIA-PROSEAL: AN IN VITRO STUDY

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ABSTRACT
Sealing of the apex and sealers that fit close to dentin are paramount in the success of endodontics in the long term and in preventing microleakage. This study compared the ability of the apical sealing ability and the dentin-sealer interfacial adaptation of AH Plus and Dia-ProSeal. In the present in vitro study, the number of extracted single-rooted mandibular premolars was 90. Sixty specimens were assigned for dye-penetration analysis, which included the groups of AH Plus, Dia-ProSeal, positive-control and negative-control. The remaining 30 specimens were equally split and evaluated using scanning electron microscopy for dentin–sealer adaptation. Lateral condensation was used to prepare, irrigate and obturate the root canals. Apical leakage was evaluated by using 2% methylene blue dye and stereomicroscopy, whereas Scanning Electron Microscopy (SEM) was used to evaluate interfacial gaps in the coronal, middle, and apical thirds. One-way analysis of variance (ANOVA), Tukey's post hoc test and independent-samples t-tests were performed to analyse the data. AH Plus showed significantly lower mean dye penetration than Dia-ProSeal (0.95 ± 0.13 mm vs. 1.38 ± 0.12 mm; $p < 0.001$). SEM analysis also revealed significantly smaller apical, middle, and coronal interfacial gaps for AH Plus ($P<0.001$). The greatest differences occurred apically for both sealers, and the smallest differences occurred coronally. Under the experimental conditions, AH Plus showed the best apical sealing ability and dentin modification compared to Dia-ProSeal.

KEYWORDS: Dia-ProSeal; apical microleakage; dentin adaptation; scanning electron microscopy

INTRODUCTION
Effective chemomechanical debridement and three-dimensional obturation, It acts as a barrier to prevent fluids, germs, and their byproducts from passing through the canal system, is crucial to success. Gutta-percha does not adhere directly to dentin, and root canal fillings require root canal sealers to fill irregularities and accessory canals, as well as to fill spaces joining the root canal filling and the dentinal wall. They affect clinical performance by their stream, film thickness, dimensional stability, solubility, setting characteristics, adhesion, biocompatibility and antimicrobial activity. Thus, modern research not only assessed sealers based on the reduction of apical leakage but also on the interaction with dentin, and their capacity to maintain an intact interface after set (Álvarez Vásquez, 2024; Ferreira et al., 2022).
Epoxy resin-based sealers are still very much in use due to their good physicochemical properties and proven sealing ability. The low solubility, dimensional stability, appropriate working time and bond to the radicular dentin make AH Plus useful for comparisons in endodontic research. Studies have also demonstrated that changes in the composition and mixing methods of resin-based sealers can impact their mechanical and physicochemical properties, making it imperative that materials are handled carefully and standardized (Preethi et al., 2020; Rosa et al., 2024). Its bond strength and resistance to displacement have also been used in laboratory studies as a reference sealer (Chandarani et al., 2022; Donnermeyer et al., 2018).
This, combined with the development of calcium silicate-based and other bioactive sealers, has led to a greater interest in comparing newer materials with the traditional epoxy resin compositions. Some newer sealers have bioactivity, alkaline pH and favourable biological responses; however, sealing ability, solubility, flow and interfacial properties of these sealers differ among products and test methods (Lim et al., 2020; Kwak et al., 2023). A comparative study has shown that the sealing ability of epoxy resin and bioceramic sealers is different, and neither sealing ability alone is enough to predict overall sealing ability (Asawaworarit et al., 2020; Kaul et al., 2021). Laboratory data are also useful in elucidating the mechanisms of the effects of the materials used in sealer-based obturation techniques, and clinical evidence has demonstrated that these techniques can lead to acceptable outcomes of treatment (Kim et al., 2022).
The ability of the dentin–sealer interface to adapt is especially significant since gaps between the two could serve as pathways for fluid flow and microorganisms. Although penetration of dentinal tubules can allow for micromechanical

retention, this is not a sufficient condition for interfacial adaptation. Measurable tubular penetration and marginal contact differences between AH Plus and other sealers have been shown in Scanning Electron Microscope-based investigations (Caceres et al., 2021). Other studies using systematic review methodology have also demonstrated that dentinal anatomy, irrigant use, removal of the smear layer, obturation technique, and physicochemical properties of the material can also affect sealer penetration (Ashkar et al., 2023; Seron et al., 2024). Another point of importance is the effect of sodium hypochlorite and the use of antioxidants on tubular penetration, which may affect dentin surface conditions and sealer behaviour, and change irrigation protocols (Vasudev et al., 2026).
Apical sealing is widely studied by various laboratory models, including dye penetration, fluid filtration, and microscopy, etc. All methods have their drawbacks, but the leakage assessment, when used in conjunction with the microscopic assessment, will give complementary information about both functional sealing and adaptation at the interface. Standardization of the specimen selection, canal preparation, obturation, storage, controls, and measurement procedures is crucial for getting reliable results (De-Deus et al., 2022). Research has shown that the differences in composition could result in varying leakage patterns and sealing performances (Soo-Hyuk et al., 2020). Additionally, the antibacterial and biological properties are still relevant since a good adaptation sealer should also be compatible with the periapical tissues and should have residual antimicrobial activity (Carvalho et al., 2021).
Sparse data is available on the ability of Dia-ProSeal to seal the apex and adapt to dentin in a well-controlled experimental setting. The sealing performance, in fact, as well as the sealing and interfacial behaviour of the newer sealer, may therefore be clarified by direct comparison using dye penetration and SEM.

Objectives
- To evaluate the ASA of AH Plus and Dia-ProSeal using dye penetration.
- To evaluate dentin–sealer interface adaptation at the coronal, middle and apical thirds by SEM.
- To find out if the differentiation relating the two sealers is statistically meaningful.

2. MATERIALS AND METHODS

2.1 Study Design and Sample Selection

The aim of this in vitro study was to assess the apical sealing ability and dentin adaptation of AH Plus and Dia-ProSeal. A total of 90 single-rooted mandibular premolars with mature apices and straight canals were chosen. Teeth that had any of the following characteristics were not included: teeth with cracks, teeth with previous endodontic therapy, teeth with open apices, teeth with severe curvature, or resorption lesions. Sample size was determined with G*Power with 15 specimens in each group.

2.2 Specimen Preparation

After being cleaned, teeth were decoronated to the cemento-enamel junction and submerged in regular saline. A No. 10 k-file was used to set the working length, which was subsequently reduced by 1 mm. The canals were enlarged with the step back up to No. 50 k-file, and Gates Glidden drills were used to flare the canals coronally. The sodium hypochlorite (5.25%) was used for irrigation, followed by 17% EDTA and distilled water. The waterways were dried using the paper points.

2.3 Grouping and Obturation

The teeth were separated into four groups of 15 teeth each, which included 60 teeth subjected to dye penetration, 15 teeth in the AH Plus group, 15 teeth in the Dia-ProSeal group, 15 teeth in the positive control group, and 15 teeth in the negative control group. Thirty teeth were used for SEM evaluation, and 15 each were assigned to AH Plus and Dia-ProSeal. The gutta-percha and lateral condensation technique was used to obturate the canals. Sealers were mixed according to the manufacturer's instructions. Access cavities were filled with Cavit, and the samples were placed in the 37°C chamber at 100% relative humidity for 72 hours.

2.4 Dye Penetration and SEM Evaluation

Root surfaces were coated with nail varnish and sticky wax, and the root apex area of 1 mm was exposed for the apical leakage testing. After dipping the tests in 2% methylene blue for seven days, they were longitudinally sliced and examined under a stereomicroscope. The depth of penetration of the dye was determined in millimetres. Roots were divided lengthwise, mounted onto stubs, sputter-coated in gold and then observed in the SEM at 2000×. Gaps between the interdigitation of the tooth and the restoration were determined at the coronal third, middle third, and apical third.

2.5 Statistical Analysis

The SPSS software was used to analyse the data. The Shapiro – Wilk test, histograms and qq plots were used to check on normality. The dye penetration data were analyzed using one-way ANOVA with Tukey's post hoc test, and the SEM gap comparisons were analyzed using independent-samples t-tests. Where appropriate, non-parametric alternatives were used. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Sample Distribution

Ninety mandibular premolars were extracted. Sixty specimens (66.7%) were assessed for apical sealing ability by dye penetration, and 30 specimens (33.3%) were evaluated for dentin–sealer adaptation by SEM.

The specimens were split into 15 samples each in the AH Plus, Dia-ProSeal, positive-control, and negative-control groups for dye penetration analysis. A total of 15 specimens were used for SEM evaluation, with 15 specimens being assigned to the AH Plus group and 15 being assigned to the Dia-ProSeal group.

3.2 Apical Sealing Ability

The AH Plus group had a lower mean dye penetration than the Dia-ProSeal group. AH Plus showed a mean leakage of 0.95 ± 0.13 mm, whereas Dia-ProSeal showed 1.38 ± 0.12 mm. The positive control exhibited the greatest leakage, while no leakage was measured in the negative control. The mean apical dye penetration of the AH Plus group was lower than that of the Dia-ProSeal group, and the positive control group had the highest leakage, while the negative control group had no leakage, as presented in Table 1.

Table 1. Apical dye penetration among the study groups

Group	Description	Minimum (mm)	Maximum (mm)	Mean ± SD (mm)
Group 1A	AH Plus	0.70	1.20	0.95 ± 0.13
Group 1B	Dia-ProSeal	1.20	1.60	1.38 ± 0.12
Group 1C	Positive control	3.70	4.30	4.01 ± 0.17
Group 1D	Negative control	0.00	0.00	0.00 ± 0.00

The results of the one-way ANOVA indicated that the four groups' means differed statistically significantly ($F (3,56) = 2945.780$, $p < 0.001$). Table 2 displays the results of a one-way analysis of variance that revealed a significant difference in apical dye penetration across the four groups ($F (3, 56) = 2945.780$, $p < 0.001$).

Table 2. One-way ANOVA for apical dye penetration

Source of variation	Sum of squares	df	Mean square	F value	p value
Among groups	132.350	3	44.117	2945.780	<0.001
Within groups	0.839	56	0.015	—	—
Total	133.188	59	—	—	—

AH Plus and Dia-ProSeal differed on average by 0.427 mm ($p < 0.001$), which was significant according to Tukey's post hoc test. Both sealers were significantly different from both positive and negative controls. Table 3 shows that there was a significant difference in dye penetration between AH Plus and Dia-ProSeal, with a mean difference of −0.427 mm ($p < 0.001$) in favour of AH Plus, as per Tukey's post hoc analysis.

Table 3. Pairwise comparison of apical dye penetration

Comparison	Mean difference (mm)	p value
AH Plus vs. Dia-ProSeal	−0.427	<0.001
AH Plus vs. positive control	−3.057	<0.001
AH Plus vs. negative control	0.950	<0.001
Dia-ProSeal vs. positive control	−2.630	<0.001
Dia-ProSeal vs. negative control	1.377	<0.001

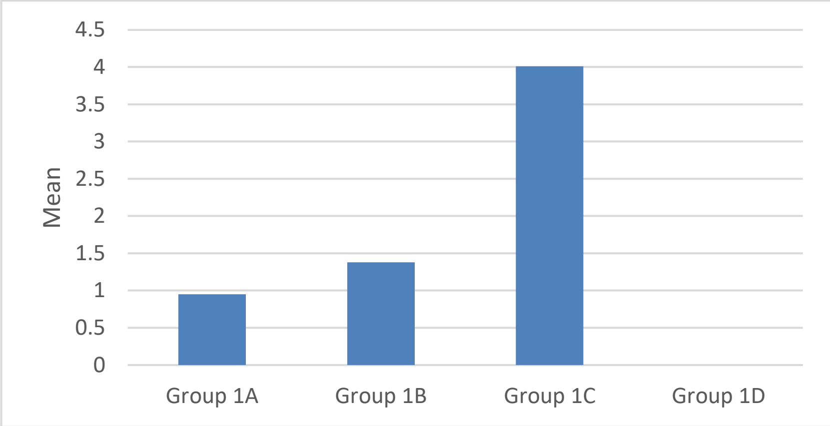

Figure 1. Mean apical dye penetration among AH Plus, Dia-ProSeal, positive-control, and negative-control groups

These data indicate that AH Plus had a lower mean dye penetration than Dia-ProSeal, as shown in Figure 1. The leakage was highest in the positive-control group, while it was absent in the negative-control group.
The representative stereomicroscopic images also indicated that less dye penetration occurred in the AH Plus specimens as compared to the Dia-ProSeal specimens.

Figure 2. Stereomicroscopic image of apical dye penetration in the AH Plus group

The mean leakage value recorded for the AH Plus specimen was less than that of the others and was confirmed by limited apical dye penetration when viewed under stereomicroscopic examination, as shown in Figure 2.

3.3 Dentin–Sealer Interfacial Adaptation

AH Plus had smaller dentin–sealer interfacial gaps than Dia-ProSeal at the apical, middle and coronal thirds. In both groups, the apical, middle, and coronal thirds had the greatest mean interfacial gap. According to descriptive results in Table 4, the dentin–sealer interfacial gaps of AH Plus were smaller than those of Dia-ProSeal at the apical, middle, and coronal thirds.

Table 4. Dentin–sealer interfacial gap measurements

Sealer	**Root third**	**Minimum (nm)**	**Maximum (nm)**	**Mean ± SD (nm)**
AH Plus	Apical	12.8	14.0	13.42 ± 0.36
AH Plus	Middle	9.4	10.1	9.78 ± 0.20
AH Plus	Coronal	6.0	6.8	6.47 ± 0.21
Dia-ProSeal	Apical	16.8	18.0	17.50 ± 0.34
Dia-ProSeal	Middle	12.3	13.0	12.70 ± 0.20
Dia-ProSeal	Coronal	9.2	9.9	9.60 ± 0.18

Independent-samples t-tests revealed that there was a statistically significant difference between AH Plus and Dia-ProSeal at all three root levels. At the apical third, the mean gap was 13.42 ± 0.36 nm for AH Plus and 17.50 ± 0.34 nm for Dia-ProSeal. In the middle third, the corresponding values were 9.78 ± 0.20 nm and 12.70 ± 0.20 nm. For the coronal third, the mean gap of AH Plus was 6.47 ± 0.21 nm while that of Dia-ProSeal was 9.60 ± 0.18 nm. Independent-samples t-tests showed that there were statistically significant differences between AH Plus and Dia-ProSeal at all three root levels; smaller interfacial gaps were found with AH Plus ($p < 0.001$), as shown in Table 5.

Table 5. Comparison of dentin–sealer interfacial gaps

Root third	AH Plus, Mean ± SD (nm)	Dia-ProSeal, Mean ± SD (nm)	t value	p value
Apical	13.42 ± 0.36	17.50 ± 0.34	−31.767	<0.001
Middle	9.78 ± 0.20	12.70 ± 0.20	−40.272	<0.001
Coronal	6.47 ± 0.21	9.60 ± 0.18	−43.828	<0.001

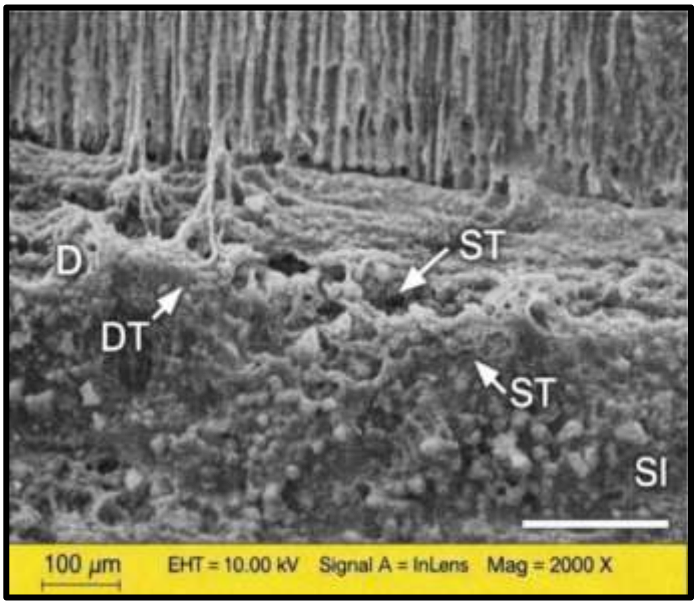

Figure 3. Comparison of dentin–sealer interfacial gaps at the apical, middle, and coronal

As seen in Figure 3, the interfacial gaps of AH Plus were smaller than those of Dia-ProSeal at all three levels of the root. The greatest differences occurred at the apical third and the least at the coronal third for both sealers.

3.4 SEM Findings

Closer and more continuous adaptation of AH Plus to the dentinal surface as observed under SEM. Dia-ProSeal has comparatively bigger interfacial gaps between the sealer and dentin.

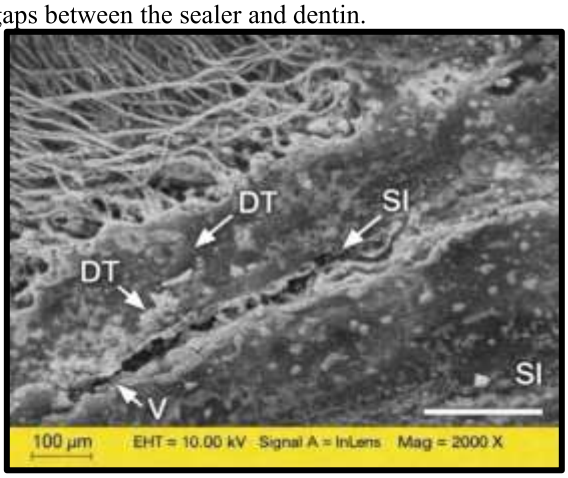

Figure 4. Representative SEM images of the AH Plus–dentin and Dia-ProSeal–dentin interfaces

As seen in Figure 4, the AH Plus specimen exhibited a greater amount of dentin–sealer contact and smaller interfacial gaps, while the specimen with Dia-ProSeal exhibited larger gaps.
All in all, apical dye leakage was significantly less and dentin–sealer interfacial gaps were significantly smaller with AH Plus than with Dia-ProSeal.

4. DISCUSSION

The current study evaluated the apical sealing capability and the dentin–sealer interfacial adaptation of AH Plus and Dia-ProSeal by using dye penetration and SEM techniques. AH Plus had significantly lower mean dye penetration than Dia-

ProSeal, and SEM analysis revealed significantly smaller inter-faces at the apical, middle and coronal thirds. The results from this study suggest that AH Plus sealing ability and dentin adaptation are superior under the standardized in vitro conditions employed in this study.
These features of the superior sealing ability of AH Plus could be associated with the epoxy resin formulation, dimensional stability, low solubility, flow properties and close interaction with radicular dentin characteristics of AH Plus. Compositions of sealers can vary, and they can impact on setting time, film thickness, radiopacity, solubility, and reaction to the biological environment, thereby compromising the integrity of the obturation interface (Ferreira et al., 2022; Kandemir Demirci et al., 2023). Resin formulation and processing may also affect the mechanical or physicochemical properties, which implies that preparation of the material can have a significant impact on clinical performance and laboratory performance of the resin, as well as consistency of both (Preethi et al., 2020; Rosa et al., 2024).
The results with the present dye-penetration are in line with the results of studies that have reported good sealing characteristics of AH Plus in comparison with other modern sealers (Trivedi et al., 2019). The results of comparison tests conducted between epoxy resin, bioceramic and silicone-based materials have shown that leakage outcomes are affected by the chemistry of the sealers, canal anatomy, obturation technique and the evaluation method (Kaul et al., 2021; Soo-Hyuk et al., 2020). This is because of the significantly reduced leakage that occurred with AH Plus, which may have been a result of its being better able to adapt to the irregularities in the canals and reduce the formation of pathways that allow dye to pass through. Leakage models should, however, be taken with a pinch of salt as laboratory measurements do not directly reflect the penetration of the bacteria or long-term clinical performance (De-Deus et al., 2022).
These results were confirmed with SEM, which showed that AH Plus had a smaller dentin–sealer gap at all root levels compared with Dia-ProSeal. Previous microscopic studies have demonstrated that AH Plus penetrates dentinal tubules and stays in close contact with conditioned dentin, though there may be variations between specimens and anatomical areas (Caceres et al., 2021; Majumdar et al., 2022). In addition, its good interfacial properties might also help in enhancing its resistance to dislodgement, given that investigations of bond strength have shown that AH Plus (Chandarani et al., 2022) can achieve good mechanical retention. However, it is important to note that penetration does not necessarily imply complete marginal adaptation as penetration depth and interfacial continuity can be different material behaviours (El Hachem et al., 2022).
The apical third and the coronal area had the greatest and least sealers, respectively, for both sealers. The pattern may be attributed to the following factors: decreased dentinal tubule density, limited access, increased complexity, and limited irrigant and sealer penetration into the apical region. However, there is systematic evidence showing that irrigation protocols, dentin condition, flow of sealer and smear-layer removal affect tubular penetration (Seron et al., 2024; Vasudev et al., 2026). An inherent difference between the two sealers was still observed, even though the use of sodium hypochlorite and EDTA in the present study may have allowed an exposure of the tubules.
In addition, temperature, moisture, and handling conditions also affect the physicochemical performance of today's modern sealers. Studies of calcium silicate materials have revealed that flow and setting properties and other factors relevant to the material can be modified under the influence of heating and environmental conditions (Mann et al., 2022; Nomura et al., 2023). There have also been significant differences found in the biocompatibility, solubility and physical characteristics of bioactive sealers in broad comparisons (Kwak et al., 2023; Wuersching et al., 2022). These results justify a case study of each material rather than using a "generic" approach to all sealers in a particular chemical category.
The present results supported AH Plus, but care needs to be taken when reaching clinical conclusions. In addition to sealer properties, clinical outcomes are affected by the properties of the disinfection, the canal anatomy, the quality of obturation, the coronal restoration and the host factors. However, despite laboratory differences, different sealers have yielded satisfactory clinical results and postoperative reactions (Coşar et al., 2023). Retreatment is also a consideration as the type of sealer can influence the removal of filling materials from complex canal systems (Jamleh et al., 2023).
Limits of the findings included the in vitro design, dye penetration model, destructive cross-sections, potential SEM preparation artefacts, and lack of thermal cycling, bacterial leakage testing, and long term aging. Given these constraints, AH Plus showed superior sealing at the apex and dentin adaptation than Dia-ProSeal, and both materials showed decreased adaptation at the apex in the apical third.

CONCLUSION
When tested in this in vitro study, AH Plus exhibited better apical sealing and dentin sealer–sealer interfacial adaptation than Dia-ProSeal. Means of AH Plus dye penetration are significantly less, suggesting a superior apical seal under standard experimental conditions. SEM also revealed smaller interfacial gaps at the coronal, middle, and apical thirds because of the SEM evaluation of the AH Plus, demonstrating closer adaptation to radicular dentin. The largest gaps were obtained in the third apical, which is the most inaccessible area, and the lowest density of dentinal tubules in both materials. The results of combined dye-penetration and SEM results showed that the microleakage was significantly correlated with the adaptation of the interface. The present results confirm the continued use of AH Plus as a good reference epoxy resin-based sealer and indicate that Dia-ProSeal sealing was comparable, but inferior in the present model. It is important to note, however,that because laboratory circumstances are not a replica of the biological environment, the results should not be interpreted as proof of therapeutic superiority, mechanical and thermal stresses of the oral cavity. Additional studies, including bacterial leakage, fluid filtration, micro-computed tomography, thermal ageing, and long-term clinical follow-up, are needed to validate these results. Overall, AH Plus sealed and adapted more consistently and may offer more predictable results regarding an effective seal of the root canal.

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